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Improving elms performance under drought stress: The pretreatment with abscisic acid

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ABSTRACT

Hormonal conditioning of plants in order to increase photosynthetic performance and reduce oxidative stress may improve plants' tolerance to stress. This study aims to elucidate the effects of ABA pretreatment on the photosynthetic apparatus and antioxidant battery of *Ulmus minor* plants under well watered (WW) and drought stress (DS) conditions. Leaves were sprayed with ABA (50 and 100 μM). After 25 days of treatment DS was initiated by withholding water for 6 days. Water deficit decreased the RWC, induced stomatal closure and impaired net CO_2 assimilation rate (A). However, independently of the water regime, ABA pretreatment increased plant DW accumulation, A, carotenoids and Chl a contents and reduced water loss. DS induced oxidative stress, but ABA application increased DS tolerance by the enhancement of the antioxidant system. Under WW conditions, the benefits of ABA application in reducing the cell membrane damages were noticeable. ABA pretreatment and DS induced changes in *U. minor* cell cycle of leaf cells, with a delay in S phase and an increase of FPCV coefficient. We propose that ABA pretreatment improves plant performance by increasing plant DW accumulation and augmenting the antioxidant system of *U. minor* plants, not only under DS conditions, but also under WW conditions. The use of ABA as pretreatment to alleviate the negative effects of DS seems to be a promising strategy to reduce plant's water loss and improve plant productivity in drought prone habitats.

1. Introduction

Water deficit is one of the main environmental stress factors that negatively affect plant growth and final yield performance of a crop. Due to the global climate changes, world water scarcity is increasing. It is estimated that up to 45% of the world agricultural lands are already exposed to continuous or frequent drought (Ashraf and Foolad, 2007; IPCC, 2007). Unfortunately, according to the climate changes predictions this scenario is expected to increase by the end of this century (IPCC, 2007). Hence, the knowledge of physiological and biochemical mechanisms that are negatively affected by drought stress (DS) as well as the plant's strategies to mitigate those effects deserve considerable attention (e.g., Slama et al., 2007).

In plants, low water availability gives rise to several physiological and biochemical responses. The loss of turgor and osmotic adjustment decrease leaf water potential and induce stomatal closure. Consequently, the limitation of gas exchange reduces transpiration and photosynthesis, ultimately limiting plant growth and development (Shao et al., 2008). As the key process of primary metabolism, photosynthesis plays a key role in plant performance under DS conditions (e.g., Brito et al., 2003; Sperdouli and Moustakas, 2012). When net photosynthesis decreases, the excess of excitation energy in the photosystem II (PSII) leads to an impairment of photosynthetic function and to an accumulation of reactive oxygen species (ROS) causing oxidative damages in plants (Wilhelm and Selmar, 2011). ROS, such as $O_2^{\bullet -}$, H_2O_2 and OH radicals, can directly attack the membrane lipids, inactivate enzymes and damage the nucleic acids leading, in many cases, to cell death (Azevedo et al., 2005; Monteiro et al., 2012). To cope with oxidative stress, plant cells developed a highly efficient defense system, with both antioxidant enzymes (e.g., SOD, CAT) and antioxidant metabolites (e.g., ascorbate) that can neutralize free radicals and reduce the potential damages of ROS. The accumulation of some organic

Abbreviations: A, net CO_2 assimilation rate; ABA, abscisic acid; APX, ascorbate peroxidase; C, group of plants under WW condition; C50, group of plants treated with 50 μM ABA under WW condition; C100, group of plants treated with 100 μM ABA under WW condition; CAT, catalase; C_i, intercellular CO_2 concentration; Chl, chlorophyll; DS, drought stress; DW, dry weight; E, transpiration rate; F_0, minimum Chl fluorescence; FCM, flow cytometry; F_m, maximum Chl fluorescence; F_v, variable fluorescence; F_v/F_m, maximum quantum yield of PSII; g_s, stomatal conductance; JA, jasmonic acid; JA-Ile, jasmonyl-isoleucine; PI, propidium iodide; MDA, malondialdehyde; RWC, relative water content; S, group of plants under DS condition; S50, group of plants treated with 50 μM ABA under DS condition; S100, group of plants treated with 100 μM ABA under DS condition; SA, salicylic acid; SOD, superoxide dismutase; WW, well watered.

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compatible compounds, such as L-proline, plays a significant role in plant protection and adaptation to a broad range of stress (Ashraf and Foolad, 2007; Dias et al., 2013a). Besides its important role as an osmolyte for osmotic adjustment, proline also contributes to the detoxification of ROS, protection of membrane integrity and stabilization of enzymes/proteins (Ashraf and Foolad, 2007). Several phytohormones, such as abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and jasmonyl-isoleucine (JA-Ile, the most biologically active form of jasmonates) (Fonseca et al., 2009), are pivotal for plant growth and development, but also play an important role in integrating several stress signals and controlling downstream stress responses (Corcuera et al., 2012).

The phytohormone ABA is well known as one of the main internal plant signals that play a critical role in the regulation of plant responses from the whole plant level to the cellular level (Efetova et al., 2007). ABA regulates the plant's adaptive response to a wide range of environmental stresses, such as DS, via diverse physiological and developmental processes. Under DS conditions, ABA primarily promotes stomatal closure to minimize water loss by transpiration and then mitigates stress damage through the activation of many stress-responsive genes that collectively increase plant stress tolerance (Aroca et al., 2008). In particular, this phytohormone interacts with membrane phospholipids to stabilize the membranes under stress conditions and enhances plant tolerance to oxidative stress by increasing the activity of antioxidant enzymes (Guschina et al., 2002). The stress-related responses of plants by ABA usually occur before any change of plant water status during soil drying and are considered the first line of defense as soil water deficits starts (Liu et al., 2005).

The effects of ABA on cell cycle have been studied mostly in roots and in in vitro cells, supporting that ABA inhibits DNA replication and cell division, which results in retarded plant growth. Cell cycle changes were described in e.g., *Pisum sativum*, *Nicotiana tabacum* or *Zea mays* after ABA treatment (Levi et al., 1993; Muller et al., 1994; Swiatek et al., 2002). ABA has been shown to be involved in promoting drought tolerance through the enhancement of the antioxidant system by exogenous application in several species (e.g., Duan et al., 2007; Wang et al., 2011). However, at the photosynthetic level little is known, and the available data demonstrates that *A* values increased in *Phaseolus vulgaris*, *Beta vulgaris*, *N. tabacum* and *Z. mays* plants previously treated with ABA (before the onset of DS) (Pospíšilová and Batková, 2004). Trouverie et al. (2003), Ma et al. (2008), Li et al. (2004), Yin et al. (2004), Duan et al. (2007) and Wang et al. (2011) also studied the effects of exogenous ABA application but, in their studies, the onset of the ABA treatment coincided with the DS treatment. These authors found that the simultaneous exposure to DS + ABA negatively affected plant height, biomass accumulation and *A* in *Malus domestica*, *Populus davidiana*, *Populus kangdingensis*, *Populus cathayana*, *Picea asperata* and *Actinidia deliciosa*. This raised a question if, instead of simultaneous treatment, a preconditioning of plants with ABA might improve their tolerance to DS.

Elms (*Ulmus* sp.) are widely used as ornamental and as timber source. Among other ornamental woody species, elms present a moderate tolerance to DS, for example, being considered to be more drought tolerant than willows or cottonwoods. Within this genus, the *U. minor* species is an elegant model in studying biotic and abiotic stresses (e.g., Oliveira et al., 2009; Conde et al., 2008; Dias et al., 2011, 2013b). Moreover, Solla and Gil (2002) described that DS could influence the development of Dutch Elm Disease symptoms in this species, with consequences on elm resistance and breeding (Desprez-Loustau et al., 2007). Therefore, ABA pretreatment to prevent/reduce plant loss under DS conditions could be a promising strategy that deserves to be further investigated.

We propose here that ABA must be applied prior the stress imposition (pretreatment), and not during the stress, in order to allow the plant to trigger defense mechanisms necessary to deal with stress. We also hypothesize that some of these key mechanisms involve improved photosynthesis and oxidative protection. We tested this hypothesis of ABA-pretreatment, using elms and we analyzed several parameters: plant growth (DW accumulation), RWC, photosynthesis, pigments, H_2O_2, phytohormones, proline, antioxidant enzymes, lipid peroxidation and cell membrane stability. Considering the contribution of cell division to plant growth we also analyzed the effects of ABA on cell cycle and on ploidy levels. We demonstrate here that ABA pretreatment improved plant's photosynthesis and antioxidant system, and ultimately improved plant growth.

2. Material and methods

2.1. Plant material and experimental conditions

U. minor Mill. seedlings were grown in 500 cm^3 pots containing an autoclaved mixture of peat and perlite (3:2, v/v) in a controlled climate chamber (Phytotron, Snijders, Tilburg) with a photoperiod of 16-h, a temperature of $22 \pm 2\,^{\circ}C$ and a photosynthetic photon flux density of $200 \pm 10\ \mu mol\ m^{-2}\ s^{-1}$. Two month old plants with average values of 48.4 ± 6.5 g DW (corresponding to plants with 10.0 ± 2.1 cm of stem height with 7–9 developed leaves) were randomly separated in three groups: in Group 1 – each plant was sprayed with 50 ml of distillated water; in Group 2 – each plant was sprayed with 50 ml of 50 μM ABA; and in Group 3 – each plant was sprayed with 50 ml of 100 μM ABA. ABA application was performed twice a week during 4 weeks.

After 25 days of ABA treatment, two water regimes were employed in the three groups: well watered (WW) and drought stress (DS). In the WW treatment the pots were watered at 100% field capacity (FC) by replacing the amount of water transpired every second day (water loss was measured by weighing the pots). In the DS treatment, water was withholding for 6 days.

For the water regime experiments, each Group (1, 2 and 3) was separated in two sub-groups with 20 plants each. The plants of the Group 1 were randomly separated in two sub-groups, C and S (C: plants previously sprayed with water under WW condition; and S: plants previously sprayed with water under DS condition); the plants of Group 2 were randomly separated in two sub-groups, C50 and S50 (C50: plants previously sprayed with 50 μM ABA under WW condition; and S50: plants previously sprayed with 50 μM ABA under DS condition); and the plants of Group 3 were randomly separated in two sub-groups, C100 and S100 (C100: plants previously sprayed with 100 μM ABA under WW condition; and S100: plants previously sprayed with 100 μM ABA under DS condition). After 6 days under WW and DS conditions, the RWC, gas-exchange parameters, chlorophyll *a* fluorescence, cytometry analysis, Rubisco activity, cell membrane permeability were analyzed. At the same time, leaf samples were collected, frozen immediately in liquid nitrogen and stored at $-80\,^{\circ}C$ for further quantification of pigments, phytohormones, proline, antioxidant enzymes, H_2O_2 content and MDA.

2.2. Plant water status and plant growth

Plant water status was assessed through the determination of the RWC of leaf discs. RWC was calculated as $100 \times (FW - DW)/(TW - DW)$, where FW is the fresh weight of leaf discs, TW is their turgid weight (determined after floating for 180 min leaf discs on water at $5\,^{\circ}C$) and DW is the dry weight (determined after drying the leaf discs at $80\,^{\circ}C$ for 48 h). Total plant DW